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Bioorganic & Medicinal Chemistry 12 (2004) 1713–1730
Bioorganic & Medicinal Chemistry

SSR182289A, a selective and potent orally active thrombin inhibitor

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Received 1 August 2003; accepted 13 January 2004

Abstract—SSR182289A 1 is the result of a rational optimisation process leading to an orally active thrombin inhibitor. The structure incorporates an original 2-(acetylamino)-[1,1′-biphenyl]-3-sulfonyl N-terminal motif, a central L-Arg surrogate carrying a weakly basic 3-amino-pyridine, and an unusual 4-difluoropiperidine at the C-terminus. Its synthesis is convergent and palladium catalysis has been employed for the construction of the key C–C bonds: Suzuki coupling for the bis-aryl fragment and Sonogashira reaction for the δ–ε bond of the central amino-acid chain. The compound is a potent inhibitor of thrombin's activities in vitro and demonstrates potent oral anti-thrombotic potencies in three rat models of thrombosis. The observed in vitro potency could be rationalized through the examination of the interactions within the SSR182289A **1** - thrombin crystal structure. SSR182289A **1**, has been therefore selected for further development.

1. Introduction

The key position of thrombin in the blood coagulation cascade together with its effects on platelet aggregation or smooth muscle cell proliferation have rendered this enzyme a natural target for antithrombotic intervention. Thrombin is the serine protease that cleaves fibrinogen to fibrin monomers, which, after polymerisation, form the central core of blood clots.[1] This proteolysis occurs adjacent to basic residues but more specifically, arginyl peptide bonds.[2] This recognition element has been therefore used as a template for the design of inhibitors. Historically, impressive synthetic work starting from two simple lead compounds TAME (*N*-Tosyl-L-Arginine Methyl Ester)[3a] and benzamidine[3b] resulted in the development by two independent groups of Argatroban (MD 805)[4] **2** and NAPAP[5] **3**, described as steric inhibitors (Scheme 1). Additionally, a transition-state strategy from the sequence of fibrinogen has resulted in the tripeptide arginal GYKI 14766[6] **4**, described as the first oral thrombin inhibitor.[7] From these compounds, enormous efforts have been dedicated over the last decade towards the design of potent and specific thrombin inhibitors, the ultimate goal remaining oral activity.[8] Indeed, the limitations associated with the clinical use of anticoagulants such as warfarin, heparin and hirudin, together with the efficacy of active site inhibitors of thrombin in animal models of thrombosis[9] has highlighted the need for orally active thrombin inhibitors. Ximelagatran[10] **5b**, the double pro-drug of Melagatran[11] **5a**, represents the most advanced oral drug candidate studied in clinical trials. We would like to report here our contribution to the field that is, the identification of SSR182289A 1 from a rational drug design process (Scheme 2), its convergent synthesis, its key in vitro

Keywords: SSR182289A; Thrombin inhibitor; L-arg surrogate; Oral antithrombotic.
Abbreviations: DMF, dimethylformamide; THF, tetrahydrofuranne; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; HBTU, O-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; TBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate.
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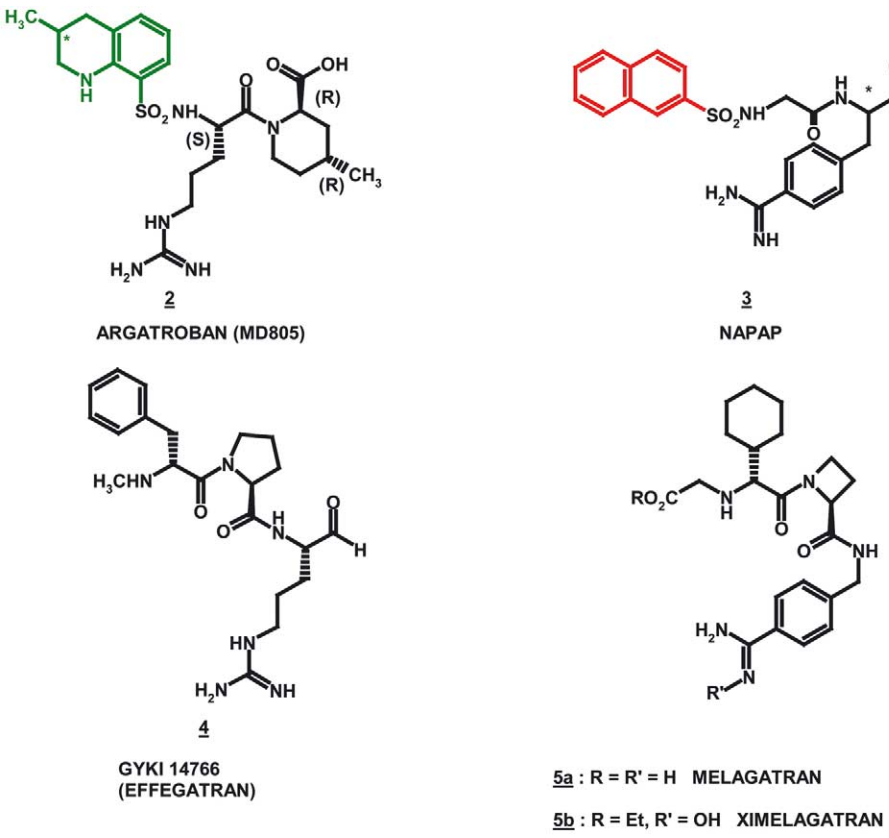
doi:10.1016/j.bmc.2004.01.016



Scheme 1.

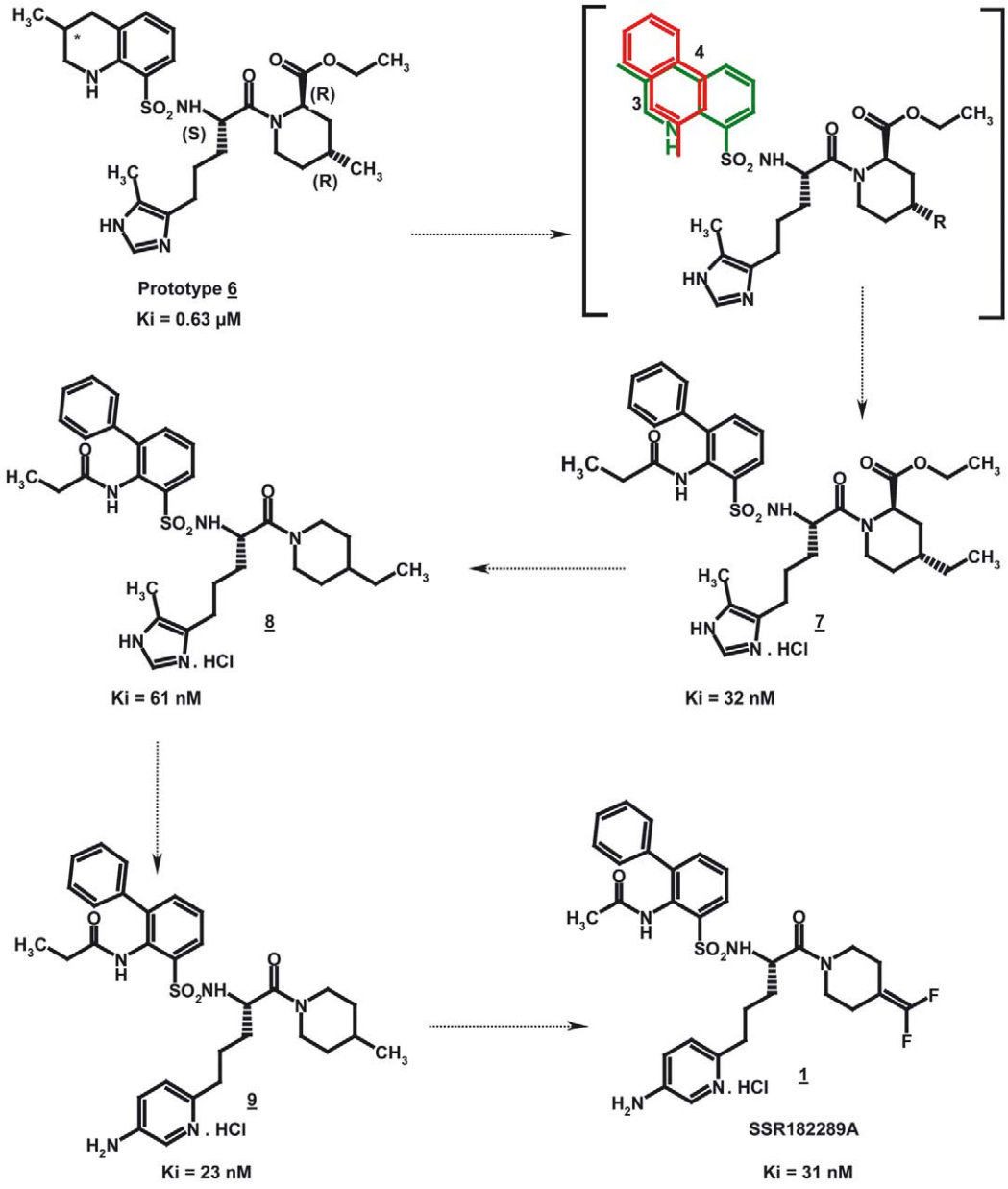
properties[12] as well as in vivo activities in rat models of thrombosis,[13] and its interactions with the target enzyme in the crystalline state.

2. Chemistry

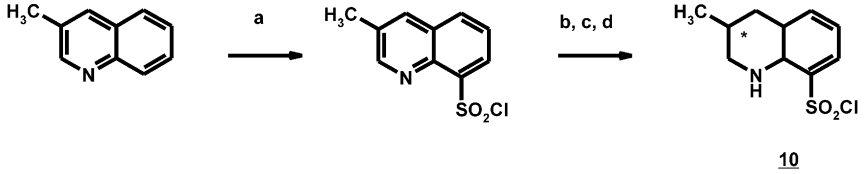
Schemes 3–8 describe the synthesis of compounds **6**, **7**, **8**, **9** considered as important in the optimisation process leading to SSR182289A **1** and which therefore are representative of the chemistry developed for this programme.

For the synthesis of the prototype **6**, we decided to modify the original synthesis[14] with hydrogenolysis at the final step in order to increase the versatility of the approach that is, potential modulations of the chlorosulfonyl and central amino-acid fragments. A simple retrosynthetic analysis highlighted the chlorosulfonyl derivatives **10** (Scheme 3) and the arginine surrogate **12** (Scheme 5) as key intermediates. Commercially available 3-methylquinoline was treated at 140 °C with chlorosulfonic acid to provide the 8-chorosulfonyl derivatives as the expected major regioisomer. The crude 8-chlorosulfonyl derivative was hydrolysed in aqueous medium and then hydrogenated over 5% rhodium on carbon. After washing in hot water, the racemic sulfonic acid was obtained in pure form and free from over-reduction products. The Widlanski's procedure[15] was used for the tedious chlorination step of the sulfonic acid in the presence of the secondary aniline and the expected intermediate **10** was isolated as a yellowish oil.

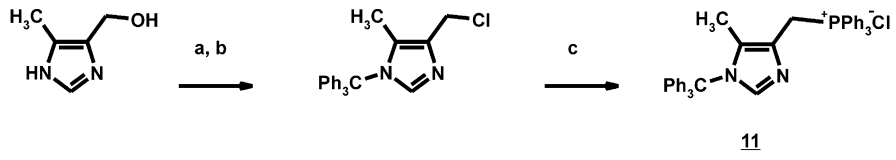
For the synthesis of **12**, we originally planned the creation of the γ–δ bond for which the Wittig reaction appeared appropriate. 4-Hydroxymethyl-5-methyl imidazole (Scheme 4) was *N*-protected as a classical trityl group which appeared suitable for both mild electrophilic and nucleophilic conditions. Using triethylamine in DMF, both τ and π isomers were obtained, the former being the major isomer. The crude mixture was used in the chlorination step[16] and the resulting chloromethyl derivative was rapidly isolated as a white powder. Indeed, the chloromethyl derivative appeared unstable on standing in concentrated solution (CH_2Cl_2) but stable when isolated as a powder. Finally, the phosphonium salt **11** was obtained by heating with triphenylphosphine in DMF solution followed by removal of the solvent and trituration with ether or, directly, by heating with triphenylphosphine in benzene, followed by filtration of the resulting precipitate after cooling. For the aldehyde partner of the Wittig reaction (Scheme 5), the choice of the protecting groups was important: the Cbz was introduced at the N-terminus with the aim of being removed within the same operation required for the saturation of the Wittig olefin, the *t*-butyl group at the C-terminus was chosen to prevent lactonization of the γ-alcohol, precursor of the aldehyde function. The aldehyde was therefore prepared in six steps from inexpensive L-aspartic acid using a published procedure.[17] The phosphonium salt **11** was deprotonated in THF at low temperature with n-butyllithium and the resulting dark orange solution of the phosphorane was transferred with a canula to a cooled solution of the aldehyde in THF to minimize potential racemization.



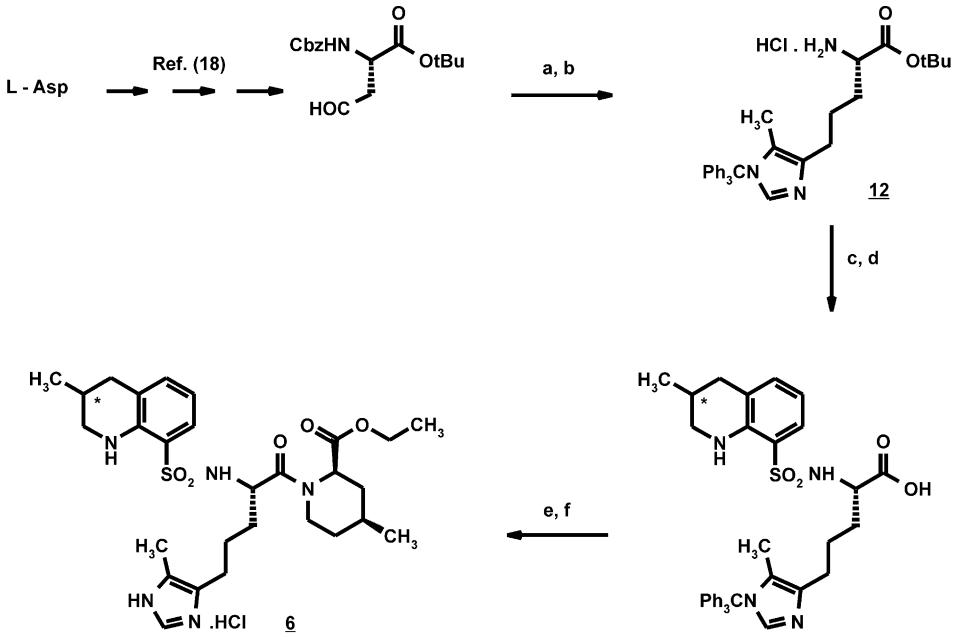
Scheme 2.



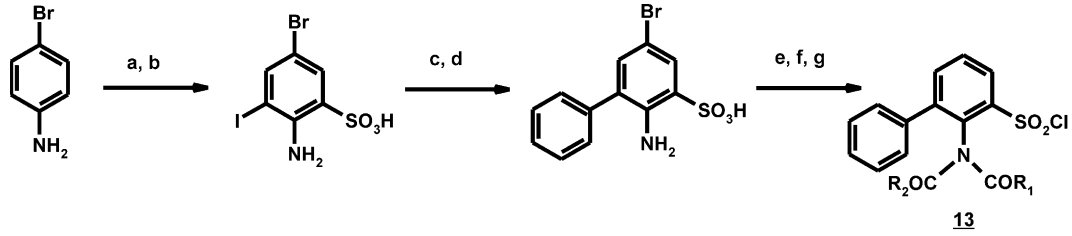
Scheme 3. (a) HSO_3Cl, 140 °C, overnight, then water, 96%; (c) H_2, Rh-C 5%, water-12N HCl, 70 °C, 16 h, 69%; (d) NEt_3, CH_2Cl_2, 0 °C, then PPh_3/SO_2Cl_2, CH_2Cl_2 from 0 °C to rt, 1 h, 93%.



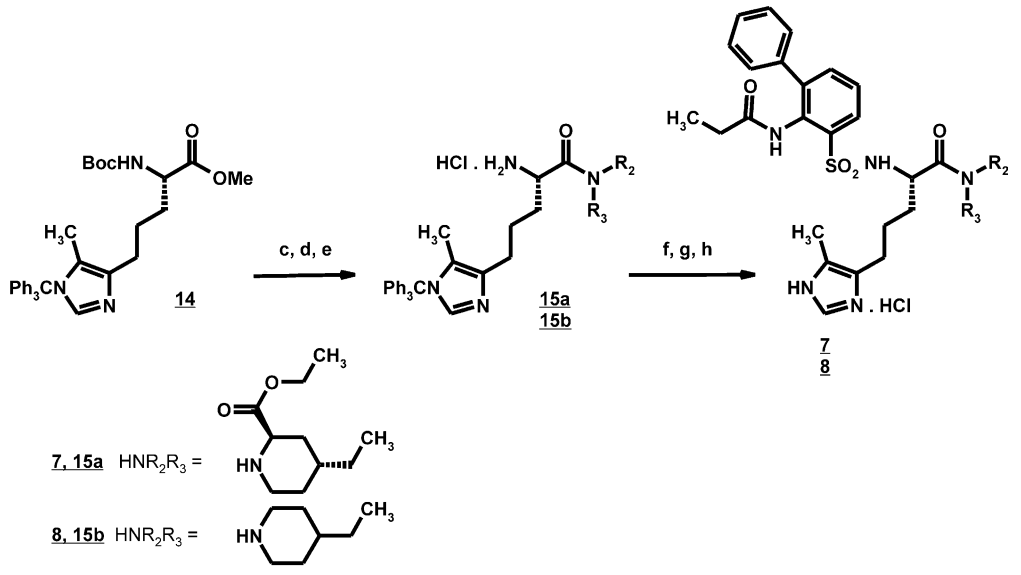
Scheme 4. (a) Ph_3Cl, Net_3, DMF, 0 °C, 3 h, 73%; (b) $SOCl_2$, CH_2Cl_2, cat. DMF, 0 °C, 5 h, 100%; (c) PPh_3, DMF, 80 °C, 5 h, 72%.



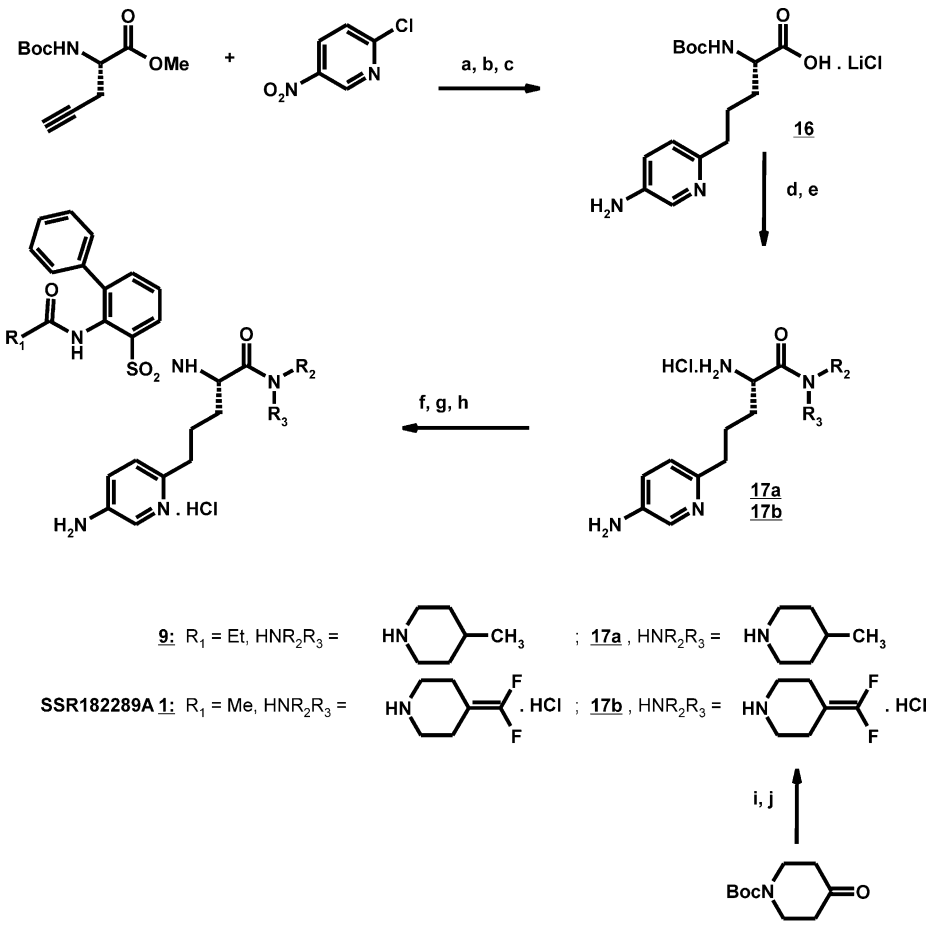
Scheme 5. (a) **11** nBuLi 1.6 M hexanes, THF −70 °C to rt, 2 h, 50%; (b) H2, Pd-C 10%, EtOH, rt, 50 psi, 7 h, 78% then HCl 1M (1 equiv) iPrOH; (c) NEt_3, **10**, CH_2Cl_2, 0 °C, 1 h 30, 91%; (d) HCl gas, C_6H_6, 5 °C, 15 min, rt, 86%; (e) BOP, DIEA, (2R,4R)-4-methyl-2-piperidine-carboxylic acid ethyl ester chlorhydrate, CH_2Cl_2, 0 °C, 2 h, rt, overnight, 99%; (f) AcOH-THF-H_2O, 90 °C, 5 h, 74% then HCl 1N iPrOH..



Scheme 6. (a) conc. H_2SO_4 (1.2 equiv), dichlorobenzene, 180 °C, 6 h; (b). ICl (1.6 equiv), MeOH, aq, HCl 1N, 90 °C, 18 h (2 steps) 60%; (c) Ph $B(OH)_2$ (1.6 equiv), $Pd(PPh_3)_4$ (5%), Na_2CO_3 (3 equiv) DME-H2O 2:1, reflux, 4 h; (d) conc. H_2SO_4 (1.1 equiv) MeOH-HCl 1N 1:1, 60% (2 steps); (e) Pd-C 10%, 50 psi, EtOH-AcOH 1:2.5, 50 °C, 66%; (f) NEt_3 (1.2 equiv), $(R_1CO)_2O$ (20 equiv), 150 °C, 16 h, 81% for $R_1 = R_2$ or $(R_1CO)_2O$ (6.5 equiv), 80 °C, 2 h, 94% followed by NEt_3 (1.5 equiv), $(R_2CO)_2O$ (10 equiv), CH_2Cl_2, reflux, 3 h, 100% for $R_1 \# R_2$; (g) PCl_5 (1.3 equiv), CH_2Cl_2, rt, 4 h, 47% for $R_1 = R_2$ or PCl_5 (1.5 equiv), CH_2Cl_2, reflux, 3 h, 60% for $R_1 \# R_2$



Scheme 7. (c) 1N NaOH (1.2 equiv), MeOH, rt, 24 h, 100%; (d) HNR_2R_3 (1.1 equiv), HBTU (1.1 equiv), DIEA (2.6 equiv), CH_2Cl_2, rt, overnight, 79%; (e) HCl gas, benzene, 0 °C, 2 h, 92% for **15a**, 85% for **15b**; (f) **13** NEt_3 (excess), CH_2Cl_2, rt; (g) AcOH-EtOH, 2:1 mixture, reflux, for $R_1 \# R_2$; (h) HCl 0.1 M iPrOH, reverse phase chromatography, 61% for **7**, 47% for **8**.



Scheme 8. (a) $PdCl_2(PPh_3)_2$ (0.04 equiv), CuBr (0.05 equiv), DIEA (2 equiv), CH_2Cl_2, 40 °C, 1 h 30; (b) HCO_2NH_4 (10 equiv), Pd-C (10%), MeOH, reflux, 2 h 30; (c) LiOH (1.1 equiv), MeOH-H_2O, rt, overnight, 72% (3 steps); (d) HNR_2R_3, TBTU (1.1 equiv), DIEA (2.6 equiv), CH_2Cl_2-DMF; (e) HCl gaz, Ch_2Cl_2, 86% for **17a**, 96% for **17b** (2 steps); (f) **13**, NEt_3 (excess), CH_2Cl_2; (g) NH_3 gas, THF, 0 °C to rt; (h) HCl 0.1 M, iPrOH, reverse phase chromatography, 69% for **9**, 46% for **1** (3 steps); (i) CF_2Br_2 (2 equiv), HMPT (4.2 equiv), triglyme, 80 °C, 2 h, 61%; (j) HCl gas, CH_2Cl_2, 0 °C, 2 h, 83%.

The *E* isomer was the major isomer as shown in the NMR spectrum of the crude material but the Wittig reaction proceeded in moderate yield (45% overall). Hydrogenation was carried out over palladium on charcoal in ethanol and the key amine **12** was isolated as the hydrochloride salt. The sulfonylation step was performed with **10** and excess triethylamine in dichloromethane at 0 °C. Regioselective *t*-butyl ester deprotection was achieved with gaseous hydrochloric acid in benzene without affecting the trityl group. The tertiary peptide link was created using the Castro reagent and the hydrochloride salt of (2*R*,4*R*)-4-methyl-2-piperidine carboxylic acid ethyl ester which was obtained through the reported six steps sequence.[18] Final deprotection of the trityl group was obtained using equivolumes of water–THF–acetic acid at 90 °C. The base was purified over silica gel and the hydrochloride was purified again by reverse phase chromatography to ensure optimum chemical purity of compound **6** obtained as a 50:50 mixture of diastereoisomers.

For the synthesis of compounds **7** and **8** (Scheme 7), we had to develop new syntheses[19] for the unknown bis-aryl chlorosulfonyl derivative **13** (Scheme 6). Intermediate **13** was therefore prepared in 7 steps from 4-bromo-aniline. The bromine atom has been utilized to block the electrophilic para position and consequently re-orient the new electrophilic reactions to *ortho-ortho′* positions. The sulfonation was performed with one equivalent of concentrated sulfuric acid in refluxing dichlorobenzene[20] and the iodination with ICl in aqueous acidic methanol solution at 90 °C. The resulting iodo derivative was submitted to Suzuki coupling[21] with phenyl boronic acid using palladium tetrakis(triphenylphosphine) as the catalyst and aqueous sodium bi-carbonate as a base in a DME-water mixture. The sulfonic acid was restored on simple treatment with one equivalent of concd sulfuric acid in a 1:1 mixture of methanol and 1 equivalent of 1N aqueous chlorohydric acid solution. The protective bromine atom was readily removed by hydrogenolysis by exposure to 50 psi of hydrogen on 10% palladium on charcoal or, alternatively, with zinc in basic media. The imides (symmetrical or unsymmetrical) were found to be viable precursors of the amides, as a NH-blocking group was necessary for the chlorination step. Treatment of the triethylammonium salt with excess anhydrides followed by standard treatment with phosphorus pentachloride[22] in dichloromethane provided the stable intermediate **13** in modest yields (47–60%). The unsymmetrical imides

carrying an acid-labile acyl group were found to be more convenient for their concomitant removal with the trityl group at the ultimate step of synthesis of the imidazolyl-containing compounds.

Classical ester hydrolysis of the *N*-Boc derivative **14**, amide formation with the required secondary amines using the more efficient HBTU reagent and regioselective acidic Boc-deprotection afforded the dipeptide coupling partners **15a,15b** (Scheme 7). As mentioned above, the sulfonylation with intermediate **13** took place at 0 °C with triethylamine as a base. Treatment in refluxing 2:1 mixture of acetic acid water provided the final compounds **7** and **8**, for which the bases were purified over silica gel and purified again as their hydrochlorides on reverse phase chromatography.

Scheme 8 depicts the syntheses of the related compounds **9** and SSR182289A **1**. A more versatile approach has been developed for the synthesis of the amino-acid derivatives bearing amino-pyridines and related heterocycles, involving a Sonogashira coupling.[23] The *N*-Boc-L-propargyl methyl glycinate was coupled with reactive 2-chloro-2-bromo-5-nitro-pyridine in dichloromethane, using copper bromide to reduce propargylic homocoupling and a temperature below 40 °C to minimize the formation of the unstable allenic isomer of the formed propargyl intermediate. The propargylic adduct as well as the nitro group were reduced concomitantly under hydrogenation transfer conditions using ammonium formate in refluxing methanol. Ester hydrolysis was achieved using lithium hydroxide to afford the acid **16** as a 1:1 adduct with lithium chloride. The peptide bond is formed using TBTU and the required 4-substituted-piperidines. In the case of SSR182289A **1**, the hydrochloride salt of 4-difluoromethylene-piperidine was sequentially obtained through a Wittig type reaction using dibromodifluoromethane and HMPA in excess to generate first the phosphonium salt and then the corresponding ylide.[24] Standard *N*-Boc deprotection was then obtained using bubbling hydrochloric acid in dichloromethane. Similarly, the chlorohydrate salt of intermediate **17** was obtained under the same conditions. The sulfonylation was performed using the conditions described previously in Scheme 7. Aminolysis of the imides using bubbling ammonia in THF at 0 °C afforded cleanly the expected amides. The bases were purified over silica gel and then treated with 1.1 equivalent of a 0.1N HCl solution in isopropanol. The salts were purified over reverse phase chromatography to ensure high purity of compounds **9** and SSR182289A **1**, isolated as lyophilisates. The optical purity of **1** has been determined in the laboratory through a racemisation study: a sample of 20 mg of **1** was heated at 150 °C for 16 h in methanol in the presence of a saturated solution of sodium bicarbonate. Co-injections of the racemised sample with the sample resulting from synthesis on chiral HPLC confirmed an optical purity superior to 99% with respect to the detection level. Proof of the maintenance throughout synthesis of the absolute S configuration at the α carbon centre was further provided by the resolution of the X-Ray structures of inhibitors bound in the chiral environment of thrombin (vide infra).

3. Biology

The description of the biological profile of SSR182289A **1** has been recently published[12,13] in comparison to that of Ximelagatran **5b** and of its active metabolite, Melagatran **5a**. We will emphasize here key data related to the in vitro profile of **1** (Table 1) and selected in vivo data following oral administration to rats in well-documented experimental animal models of thrombosis (Table 2).

SSR182289A **1** was a potent inhibitor of thrombin's amidolytic activity with a K_i of 31 nM (Table 1). Moreover, the compound was very selective (>5000-fold) towards other enzymes such as tPA, plasmin, APC and trypsin (>1500 fold). SSR182289A **1** was also a potent inhibitor of thrombin generation in human plasma with an IC_{50} of 150 nM and a potent anti-platelet agent

Table 1. In vitro profile of SSR182289A **1** compared to that of Melagatran **5a**

		SSR182289A 1 (μM)	**Melagatran 5a** (μM)
K_i	Thrombin	0.031	0.014
K_i	APC	>250	0.91
K_i	tPA	>250	20.7
K_i	Plasmin	>250	2.5
K_i	Trypsin	54.0	0.008
Thrombin generation in human plasma (IC_{50})		0.15	0.02
Thrombin-induced human platelet aggregation (IC_{50})		0.032	0.008

Table 2. Antithrombotic effects of SSR182289A **1** compared to those of Ximelagatran **5b**, after oral administration

	SSR182289A 1	**Ximelagatran 5b**
Rat Wessler model ED_{50} (mg/kg)	1.1	0.7
Rat AV shunt model (Tissue Factor-induced) ED_{50} (mg/kg)	3.1	5.3
Rat AV shunt model (Tissue Factor-induced)		30 min; 76%
Duration of action at 10 mg/kg.% reduction in thrombus	30 min; 51%	240 min; 28%
weight at different times after dosing.	240 min; 39%	
Rat Arterial Thrombosis model ED_{200} (mg/kg)	5.9	1.8

with an IC_{50} of 32 nM against thrombin-induced human platelet aggregation.

The antithrombotic efficacy of SSR182289A **1** following oral administration to rats is shown in Table 2. In the venous thrombosis Wessler model, SSR182289A **1** exhibited an ED_{50} of 1.1 mg/kg. In the arterio-venous shunt model in rats where thrombus formation was induced by the injection of tissue factor in the shunt, SSR182289A **1** strongly inhibited thrombus formation (ED_{50} of 3.1 mg/kg). Ximelagatran **5b** showed a similar potency. When considering the duration of action of a 10 mg/kg dose in the TF dependent AV shunt model, the antithrombotic effect of **1** was still present (39% reduction of thrombus weight) 4 h following a single oral administration, and appeared to be more persistent than that of **5b**. In the rat arterial thrombosis model where thrombus formation was induced by the application of an electrical current to the adventitial surface of the carotid artery, the oral dose of **1** required to double the time to occlusion was 5.9 mg/kg. In this model, SSR182289A **1** appeared slightly less potent than Ximelagatran **5b**.

4. Crystallisation—molecular modelling

A detailed report of the crystal structure determination of the intermolecular complex between SSR182289A **1** with thrombin at 1.5 Å resolution will be published elsewhere. Here we describe only the key interactions found between **1** and thrombin and compare them to those in the Argatroban **2**-thrombin complex. Thrombin does not seem to adjust significantly in order to accommodate the inhibitor apart from slight side chain movements relative to the structure of the enzyme without inhibitor or relative to the complex with **2** (PDB code 1DWC).[25] As anticipated, **1** adopts the hydrophobic collapse type of conformation of **2**. The amide carbonyl as well of the NH of the sulfonamide are in antiparallel H bond interactions with the canonical Gly 216.

The 3-amino-pyridine fragment of **1** lies in the deep S_1 specificity pocket of thrombin. The 3-amino group occupies the position corresponding to one of the terminal nitrogen atoms of the Arg residue found in **2**. (Fig. 1). As a consequence, it forms a hydrogen bond with a buried water molecule and one of the usual hydrogen bonds with the specificity-determining residue Asp 189. The other carboxylate oxygen of Asp 189 accepts a hydrogen bond from a nearby water molecule.

The loss of strong hydrogen bonds associated with the guanidine of **2** is not detrimental for the affinity of **1** as the 3-amino-pyridine binding to the S_1 subsite is probably compensated by important hydrophobic interactions.

Furthermore, the nitrogen atom of the 3-amino-pyridine accepts a hydrogen bond from a nearby water molecule, which at the same time donates another hydrogen bond to the carbonyl oxygen of Ser 214 and accepts one from the catalytic OH γ of Ser 195. This interaction is in sharp contrast to that reported[26] with 2-amino-pyridines where the nitrogen of the pyridine forms an interaction with Asp189 through an ordered water molecule at the bottom of the S1 site. This is the only, although indirect, connection between **1** and any residue of the catalytic triad of thrombin.

In contrast, **2** has a direct hydrogen bond to the catalytic serine of thrombin from the carboxylate moiety of the pipecolate, which sits in the S_2 subsite. The loss of this interaction in **1** did not prove essential (vide infra). The piperidine moiety of **2** is about 0.5 Å closer to the plane of catalytic histidine than that of **1** as can be seen

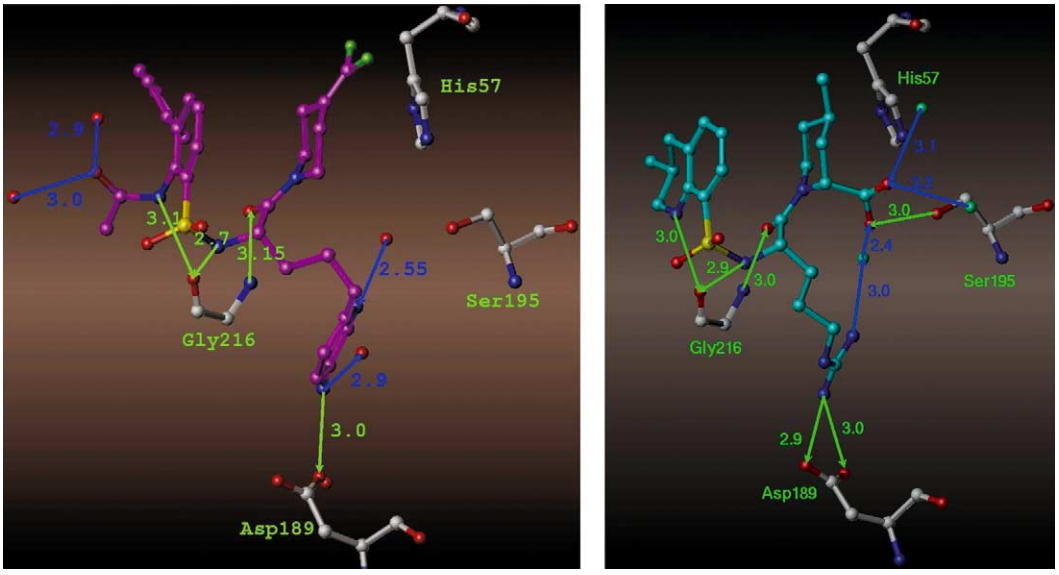


Figure 1. Hydrogen bonding pattern of the thrombin inhibitors SSR182289A **1** (left) and Argatroban **2** (right). The figure shows the direction of hydrogen donation with the bridgehead atom distances next to the arrows. A few crucial residues are labelled. Green arrows represent hydrogen bonds between protein and inhibitor, while blue ones those between inhibitor and water. Carbon atoms of SSR182289A **1** are magenta, those of Argatroban **2** are cyan, while protein carbons are white, oxygens are red, nitrogens blue, sulphurs are yellow.

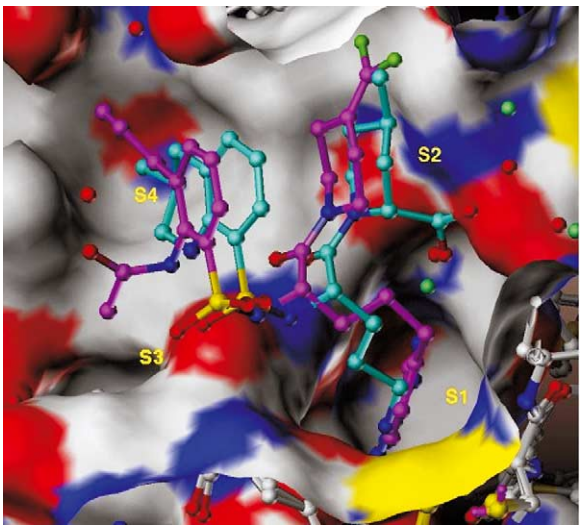


Figure 2. The picture emphasizes the spatial complementarities between protein and the two inhibitors **1** and **2** in the different subsites of thrombin. Colouring scheme is the same as on Figure 1. Note that S4 is almost completely white, emphasizing its hydrophobic nature.

in Figure 2 (the thrombin main chains of the two complexes are superimposable with an RMSD of ~0.2 Å). This is most likely due to the presence of this hydrogen bond to the catalytic serine in the complex of **2**.

In subsite S_3, it is clear that the N–H of the acetamide in **1** plays an identical role to the N–H of the 3-methyl-tetrahydroquinoline moiety in **2**, i.e., donating a hydrogen to the carbonyl group of Gly 216 which probably results in a stronger interaction in the case of **1** due to the more acidic nature of this hydrogen. However, the acetylamino group provides a methyl for the hydrophobic pocket created by the nearby Trp 215, Ile 174 and the alkyl part of Glu 217.

Additionally, the solvent exposed carbonyl group of this *N*-acetyl is engaged in 2H-bonding interactions with two adjacent water molecules (Fig. 1), providing therefore a method of stabilisation of the inhibitor–enzyme complex in that region which, to our knowledge, has not been previously described.

5. Discussion

A complete description of the process that has led to the discovery of SSR182289A **1** would certainly have required several reports. Our choice was to concentrate here on the key SAR steps in its conception. We reasoned that the presence of two highly ionised groups at the pH close to usual physiological conditions in Argatroban **2**[27] was responsible for its low aqueous solubility as well as the lack of anti-thrombotic activity following oral administration, this probably due to low membrane permeation. The replacement of the basic guanidine with a (methyl substituted) imidazolyl nucleus[28] of much reduced basicity (pK_a close to 7.5) together with the masking of the carboxylate function resulted in prototype compound **6**[29]. Although being a modest thrombin inhibitor ($K_i = 0.63$ μM), compound **6**, following oral administration to rats proved to be a reasonable anticoagulant (with a dose to double the thrombin time measured ex vivo of 5.4 mg/kg). Detailed analysis of the milestone publication[25] of D. Banner's team describing the first X-ray structures of reversible inhibitors bound to thrombin at 3.0 Å resolution enabled the design of an original achiral P_4 motif: superimposition of Argatroban **2** and NAPAP **3** in the active site revealed that the naphthyl of **3** was overlapping the 3- and 4- position of the tetrahydroquinolyl fragment of **2** (Scheme 2). High affinity could be then restored through the incorporation of a 3-bis-arylsulfonyl fragment coupled with the introduction of an N-acyl group in position 2-, mandatory for oral activity. Compound **7** maintained a level of oral anticoagulation in the same range as for compound **6** although being 20 times more potent towards the purified enzyme. Simplification of the structure was achieved through the analysis of the Mitsubishi SAR[30] showing that the (2*R*,4*R*) methyl-pipecolate portion was twice as potent (14 nM versus 30 nM) than the corresponding 4-methyl piperidine (bovine thrombin): in our case, compound **8** was also two times less potent than compound **7** (61 nM versus 32 nM, human thrombin). Further improvement in oral anticoagulant/antithrombotic activity came from the replacement of the imidazolyl nucleus by less basic amino-containing heterocycles. Work performed independantly in our laboratories to replace the P1 (methyl)-imidazole of prototype compound **6** by aminothiazoles, amino-pyrimidines, amino-pyridazines, amino-pyridines have highlighted the latter as the most promising.[31] When introducing amino-pyridines in our bis-aryl sequence, 3-amino-pyridines such as the one incorporated in compound **9** proved optimal in terms of antithrombotic performance following oral treatment. Finally, ultimate tuning of the pharmacokinetic properties of compounds such as **9** led to SSR182289A **1** isolated as a hydrochloride salt.

SSR182289A **1** exhibits a remarkable selectivity profile compared to Melagatran **5a**. The observed selectivity of **1** is probably due to the presence of the 3-amino-pyridine filling the more hydrophobic S_1 pocket of thrombin.[32] In the case of **5a**, which bears a particularly non-selective benzamidine, the problem of trypsin selectivity was circumvented by the use of a hydroxy-amidine as a bio-precursor of the amidine. The selectivity ratio of 65 observed for **5a** between thrombin and the anticoagulant enzyme APC could have, in theory, undesirable consequences if pro-drug administration leads to variable concentrations of active drug in the plasma. The higher activity of **5a** in the thrombin generation assay is probably due to the more polar nature of the compound.[33] Nethertheless, for **1**, the IC_{50} of 150 nM in human plasma seems a sufficient level of activity to anticipate anti-thrombotic efficacy. Indeed, **1** is active in three models of thrombosis in rats following oral administration. The potency of **1** is comparable to that of Ximelagatran **5b**. In the tissue factor-induced AV shunt model, when considering duration of action, the decline of activity of **5b** appears more rapid than for **1** suggesting an improved $T\frac{1}{2}$ for **1** in this species.

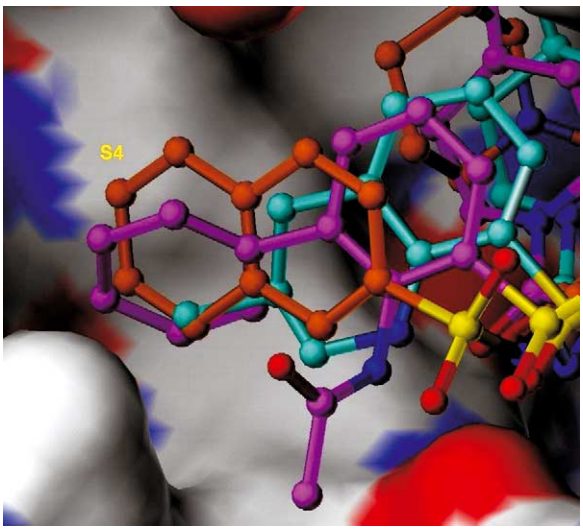


Figure 3. Superposition of the thrombin co-complex structures of SSR182289A **1** (magenta carbons), Argatroban **2** (cyan carbons) and NAPAP **3** (brown carbon atoms).

In conclusion, SSR182289A **1** is the result of an optimisation process[34] whose objective was to find a drug candidate suitable for oral administration in humans. The synthesis of SSR182289A **1** is convergent and new synthetic routes had to be developed for the synthesis of the bis-aryl chlorosulfonyl fragment and the central L-Arg surrogate (determinant for oral activity), involving in particular, palladium catalysis for the formation of key C–C bonds. The observed in vitro potency of SSR182289A **1** towards thrombin could be rationalized through the contribution of a complex network of 8H-bonds with the protein and additional water molecules, together with positive hydrophobic interactions. The crystal structure also validated the original design concept, that is the twisted bis-aryl fragment of **1** is approximately overlapping the 3- and 4-positions of the 3-methyl-tetrahydroquinolyl moiety of Argatroban **2**, thus efficiently filling the S4 subsite, as has been shown with the β-naphthyl of NAPAP **3** (Fig. 3).

SSR182289A **1** exerts potent antithrombotic effects in the rats, comparable to those of Ximelagatran in venous, mixed and arterial models of thrombosis following oral administration. Furthermore, it is also equipotent to **5b** in a rabbit venous thrombosis model.[13] In contrast to Ximelagatran, **1** does not require metabolic activation, which appears to us to be a potential advantage. Clinical studies will further define the potential of SSR182289A **1**.

6. Experimental

Melting points were determined using open capillary tubes on a Buchi 530 apparatus and are uncorrected. Merck Kieselgel 60 (230–400 μm) was used for flash chromatography and Kieselgel 60 F_{254} silica plates (0.2 mm) were used for TLC. The structures of the compounds were confirmed spectroscopically by proton and carbon NMR ($CDCl_3$, DMSO-d_6, CD_3OD or pyridine-d_5) with TMS as the internal standard using a Brucker AC-250 instrument or Brücker Avance™ 400 or 500 spectrometers, by IR on a Perkin–Elmer 297 or a FT-IT Avatar 360 (Nicolet) spectrophotomers and by their mass spectra (MS-ES) which were recorded on a VG Autospec (Fisons Instruments) spectrometers. The purity of the compounds was analyzed by TLC, by LC/MS Gilson/Micromass ZMD with a quadrupole MS equipped with a Z-spray and operating in electrospray ionisation mode, and elementary analysis. Results obtained were within ±0.4% of the theoretical values. Optical rotations were recorded using a Perkin–Elmer 343 polarimeter with a sodium source. Reagent grade chemicals were purchased from Sigma-Aldrich-Fluka. All solvents were analytical grade and anhydrous reactions were performed in oven-dried glassware under an atmosphere of argon or nitrogen.

6.1. 3-methyl-8-quinolinesulfonic acid

To a solution under argon of 55.8 mL (838 mmoles, 4 equiv) of chlorosulfonic acid is added, dropwise, at room temperature, 30 g (209 mmoles) of 3-methylquinoline. The reaction mixture is then heated at 140 °C overnight and, after cooling, poured over ice water. Water is removed under reduced pressure and the crude product is collected by filtration and washed with water. 45 g (96%) of the crude product is obtained after drying in vacuuo.

6.2. 1,2,3,4-tetrahydro-3-methyl-8-quinolinesulfonic acid

20 g (90 mmoles) of 3-methylquinoline-8-sulphonic acid is placed in a mixture of 200 mL of water and 25 mL of 12N hydrochloric acid. 6 g of 5% rhodium on charcoal catalyst is added and the reaction mixture is heated at 70 °C for 16 h. The catalyst is filtered and washed with hot water and the filtrate is evaporated to provide a residue which is dissolved in 50 mL of ethanol. After evaporation of ethanol the residue is dried over phosphorus pentaoxide at 50 °C. 14 g (69%) of product is obtained. Melting point = 255 °C (decomposition).

6.3. 1,2,3,4-tetrahydro-3-methyl-8-quinolinesulfonyl chloride, 10

To a solution of 10.46 g (40 mmoles) of triphenylphosphine in 60 mL of dichloromethane at 0 °C under nitrogen, is added 3.56 mL (44 mmoles) of sulphuryl chloride in a dropwise fashion. The temperature is then allowed to rise to room temperature before a solution of 4.56 g (20 mmoles) of 1,2,3,4-tetrahydro-3-methyl-8-quinolinesulfonic acid in 100 mL of dichloromethane and 2.78 mL of triethylamine (20 mmoles), is added over 10 min. The reaction mixture is stirred for a further hour at room temperature before being poured into 500 mL of pentane. The mixture is filtered and the filtrate is evaporated. The residue is dissolved in approximately 500 mL of pentane which is evapored once more to provide 5 g (93%) of the product in the form of an oil.

6.4. 5-methyl-1-(triphenylmethyl)-1*H*-imidazole-4-methanol

To a solution under argon of 49 g (330 mmoles) of 5-methyl-1*H*-imidazole-4-methanol hydrochloride and 91.7 mL (660 mmoles, 2 equiv) of triethylamine in 525 mL of DMF, is added, dropwise, at 0 °C, 91.7 g (330 mmoles, 1 equiv) of trityl chloride in 560 mL of DMF. Stirring is maintained for 3 h and the reaction mixture is poured over an ice-water mixture. The precipitate is filtered and washed with water followed by acetone. The white precipitate is then thoroughly dried under vacuum overnight in a phosphorus pentaoxide-equipped oven to afford 100 g (73%) of a white powder.

6.5. 4-(chloromethyl)-5-methyl-1-(triphenylmethyl)-1*H*-imidazole

To a solution of 100 g (282 mmoles) of 5-methyl-1-(triphenylmethyl)-1*H*-imidazole-4-methanol in 1.5 l of dichloromethane and 43 mL of DMF, is added, dropwise, under argon at 0 °C, 28 mL (383 mmoles, 1.36 equiv) of thionyl chloride in 150 mL of dichloromethane. Stirring is maintained for 5 h at this temperature. A saturated aqueous solution of sodium bicarbonate is cautiously added. After decantation, the organic phase is washed twice with water. The organic layer is dried over magnesium sulfate, the solvent is removed under vacuum, to afford 110 g (100%) of a crude chloromethyl derivative as a white powder.

6.6. [5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]methyl]triphenyl- phosphonium chloride, 11

To a solution of 100 g (268 mmoles) of 4-(chloromethyl)-5-methyl-1-(triphenylmethyl)-1*H*-imidazole in 600 mL of DMF, is added 85 g (305 mmoles, 1.15 equiv) of triphenylphosphine. The reaction mixture is heated to 90 °C for 5 h. The solvent is removed under reduced pressure and the crude material is taken up in a minimum volume of dichloromethane and poured over ether with stirring. Agitation is maintained for 10 h and the precipitate is filtered, washed with ether, and dried to afford 122g (72%) of the crude phosponium salt.

6.7. (2*S*,4*E*)-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-2-[(phenylmethoxy)carbonyl]amino]-,4-pentenoic acid, 1,1-dimethylethyl ester

To a suspension cooled to −70 °C of 25 g (39 mmoles, 1 equiv) of [5-methyl-1-(triphenylmethyl)-1H-imidazol-4-yl]methyl]triphenyl- phosphonium chloride in 195 mL of THF, is added, dropwise, 24.4 mL of a 1.6 M solution of *n*-butyllithium in hexanes (39 mmoles, 1 equiv). Stirring is maintained for 0.75 h at this temperature. 20 g (mmoles) of (2*S*)-4-oxo-2-[(phenylmethoxy)carbonyl]-amino]butanoic acid, 1,1-dimethylethyl ester in 25 mL of THF is rapidly added in one portion. The temperature was allowed to increase progressively, reaching room temperature after 2 h from −70 °C to room temperature, 500 mL of a saturated aqueous solution of sodium chloride in added to the reaction mixture. After decantation, the aqueous phase is extracted with ethyl acetate. The combined organic layers are washed with water and dried over magnesium sulfate. After removal of the solvent, the crude material is purified by chromatography over silica gel with ethyl acetate/hexanes 3:9 as the eluent. 0.5 g (1.3%) of the *cis* isomer and 17.5 g (44%) of the *trans* isomer are therefore obtained as pure products.

6.8. Hydrochloride salt of (α*S*)-α-amino-5-methyl-1-(triphenylmethyl)-,1*H*-imidazole-4-pentanoic acid-1,1-dimethylethyl ester 12

To a solution of 25 g (41 mmoles) of (2*S*,4*E*)-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-2-[(phenylmethoxy)carbonyl]amino]4-pentenoic acid, 1,1-dimethylethyl ester in 200 mL of ethanol, is added with caution 2 g of 10% palladium on charcoal. The mixture is hydrogenated for 7 h at 50 psi at room temperature in a Parr apparatus. After filtration over Celite and washing with ethanol, the solvent is removed under pressure. The crude material is purified by chromatography over silica gel using dichloromethane/ethanol 95:5 as the eluent. 17 g (78%) of the free base are therefore obtained and the chlorhydrate is prepared by treatment with HCl 0.1 N in isopropanol.

6.9. (α*S*)-5-methyl-α-[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-1-(triphenylmethyl)- 1*H*-imidazole-4-pentanoic acid, 1,1-dimethylethyl ester

To a solution of 2.1g (14.07 mmoles, 2.1 equiv) of 1,2,3,4-tetrahydro-3-methyl-8-quinolinesulfonyl chloride 10 in 30 mL of dichloromethane at 0 °C, is added, dropwise, a clear solution of 3.4 g (6.7 mmoles) of (α*S*)-α-amino-5-methyl-1-(triphenylmethyl)-, 1*H*-imidazole-4-pentanoic acid-1,1-dimethylethyl ester chlorhydrate 12, 1.9 mL (13.4 mmoles, 2 equiv) of triethylamine in 6 mL of dichloromethane. The reaction mixture is stirred for 1 h 30min at 0 °C. Water is then added and the aqueous phase is extracted twice by dichloromethane. The combined organic layers are dried over magnesium sulfate. After removal of the solvent under reduced pressure, the crude material is purified over silica gel using dichloromethane/methanol 99:1 as the eluent. 4.3 g (91%) of the expected product are obtained.

6.10. (α*S*)- 5-methyl-α-[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-1-(triphenylmethyl)-1*H*-imidazole-4-pentanoic acid

To a solution of 4 g (5.7 mmoles) of (α*S*)-5-methyl-α-[(1,2,3,4 - tetrahydro - 3 - methyl - 8 - quinolinyl)sulfonyl]-amino]-1-(triphenylmethyl)- 1*H*-imidazole-4-pentanoic acid, 1,1-dimethylethyl ester in 50 mL of benzene cooled at 0 °C, is bubbled chlorhydric acid for 15 min. After removal of the solvent under reduced pressure, the crude material is taken up in 50 mL of chloroform and neutralised by the addition of gaseous ammoniac. The reaction mixture is evaporated and the crude product is purified over silica gel using dichloromethane/methanol 90:10 as the eluent. Traces of methanol are eliminated through azeotropic distillation with toluene. 3.2 g (86%) of pure acid are obtained.

6.11. (2*R*,4*R*)-4-methyl-1-[(2*S*)-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxo-2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]pentyl]-2-piperidinecarboxylic acid ethyl ester

To a solution cooled to 0 °C of 1.5 g (2.3 mmoles) of (α*S*)- 5-methyl-α-[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]-1-(triphenylmethyl)-1*H*-imidazole-4-pentanoic acid in 25 mL of dichloromethane, is added 1.8 mL (11.5 mmoles, 5 equiv) of diisopropylethylamine, 0.67 g (2.53 mmoles, 1.1 equiv) of (2*R*,4*R*)-4-methyl-2-piperidinecarboxylic acid ethyl ester chlorhydrate, and finally 1.4 g (mmoles, equiv) of BOP. The reaction mixture is stirred for 2 h at 0 °C and left overnight at room temperature. The reaction mixture is evaporated to dryness and the crude material is taken up in ethyl acetate and washed successively with aqueous solutions of 1N HCl, sodium bicarbonate and brine. The organic layer is dried over magnesium sulfate and the solvent is removed under reduced pressure. The crude product is purified by chromatography over silica gel using dichloromethane/methanol 99:1. 2.15 g (99%) of the expected product is obtained.

6.12. Hydrochloride salt of (2*R*,4*R*)-4-methyl-1-[(2*S*)-5-(5-methyl-1*H*-imidazol-4-yl)-1-oxo-2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)sulfonyl]amino]pentyl]-2-piperidinecarboxylic acid ethyl ester, 6

A solution of 2.1 g (2.3 mmoles) of (2*R*,4*R*)- 4-methyl-1-[(2*S*)-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxo-2-[(1,2,3,4-tetrahydro-3-methyl-8-quinolinyl)-sulfonyl]amino]pentyl]-2-piperidinecarboxylic acid ethyl ester, in 22 mL of THF, 22 mL of water and 22 mL of acetic acid is heated to 90 °C for 5 h. After cooling, the reaction mixture is evaporated and the crude material is taken up in ethyl acetate. The organic phase is washed successively with an aqueous solution of sodium bicarbonate and brine. The organic layer is dried over magnesium sulfate. The solvent is removed under reduced pressure and the crude product is purified by chromatography over silica gel using dichloromethane/methanol 95:5 as the eluent. 0.95g (74%) of the free base is obtained. A sample of 0.37 g is treated with 6.6 mL (0.66 mmoles, 1 equiv) of an aqueous solution of HCl 0.1N and lyophilised to afford the title compound **6** as a 1:1 mixture of diastereoisomers as evidenced by HPLC analysis. Care should be taken when handling the compound which tends to aromatise slowly when exposed to daylight. Melting Point 71–75 °C, $[\alpha]_D^{20}$ +110° (*c* = 0.23; MeOH). *m*/*z* 560 $(M+H)^+$.

^{1}H NMR (500 MHz, Pyridine-d_5) δ (major conformer of the two diastereisomers): 9.50 (very broad s, 2H), 8.10–7.90 (m, 1H); 8.10–7.60 (m, 1H); 7.11 (m, 1H); 6.62 (m, 1H); 5.55–5.15 (m, 1H); 4.29 (m, 1H); 4.12 (m, 2H); 3.50–2.50 (m, 7H); 2.50–1.50 (m, 13H); 1.25–1.15 (m, 3H); 1.10–0.75 (m, 6H).

6.13. 2-amino-5-bromobenzenesulphonic acid

A mixture containing 31 g (180 mmoles) of 4-bromoaniline and 9.7 mL (220 mmoles; 1.2 equiv) of sulphuric acid in 200 mL of 1,2-dichlorobenzene is heated at 180 °C for 6 h. The reaction medium is allowed to cool to room temperature and is then filtered . The residue is washed with dichloromethane. 45 g (97%) of the product are obtained, product which is used without further purification in the following step. Melting point 240 °C.

6.14. 2-amino-5-bromo-3-iodobenzenesulphonic acid

To 45 g (176 mmoles) of 2-amino-5-bromobenzenesulphoic acid are added 46 g (282 mmoles; 1.6 equiv) of iodine chloride, 400 mL of aqueous 1N hydrochloric acid solution and 400 mL of methanol. The mixture is heated at 90 °C for 18 h and concentrated under reduced pressure and the residue is crystallized from ethanol. 41 g (62%) of product are obtained, product which is used without further purification in the following step. Melting point 240 °C (decomposition)

6.15. 2-amino-5-bromo[1,1′-biphenyl]-3-sulphonic acid

To a mixture of 37.8 g (100 mmoles) of 2-amino-5-bromo-3-iodobenzenesulphonic acid and 32 g (300 mmoles, 3.2 equiv) of sodium carbonate in 300 mL of 1,2-dimethoxyethane and 150 mL of water are successively added, under a nitrogen atmosphere, 5.8 g (5 mmoles, 0.05 equiv) of tetrakis(triphenylphosphine) palladium and 19.5 g (160 mmoles 1.6 equiv) of benzeneboronic acid. The mixture is heated at the reflux temperature for 4 h and the reaction medium is then concentrated under reduced pressure. The residue thus obtained is then dissolved in a mixture containing 300 mL of methanol, 300 mL of 1N hydrochloric acid and 16.8 mL of 95% sulphuric acid. The mixture is concentrated to 100 mL under reduced pressure, cooled to 0 °C. and filtered The residue is purified by chromatography on an RP 18 reverse-phase column, eluting with an acetonitrile:water mixture (2:8). 20 g (60%) of product are obtained after recrystallization from an ethanol/ether mixture. Melting point 197.5 °C

6.16. 2-amino[1,1′-biphenyl]-3-sulphonic acid

20 g (61 mmoles) of 2-amino-5-bromo[1,1′-biphenyl]-3-sulphonic acid is placed in the presence of 3 g of 10% palladium-on-charcoal in a mixture containing 40 mL of ethanol and 100 mL of acetic acid, in a Parr apparatus. The reaction medium is heated to 50 °C under a pressure of 0.35 Mpa (50 psi), it is filtered through Celite and the filtrate is concentrated under reduced pressure. The residue is recrystallized from an ethanol/ether mixture. 10 g (66%) of product are obtained. Melting point 241.5 °C

6.17. Triethylammonium salt of 2-[bis(1-oxopropyl)-amino]-[1,1′-biphenyl]-3-sulfonic acid

To 2.49 g (10 mmoles) of 2-amino[1,1′-biphenyl]-3-sulphonic acid is added, dropwise, 1.67 mL (12 mmoles, 1.2 equiv) of triethylamine. To the resulting mixture is added at room temperature, 150 mL of propionic anhydride. The reaction mixture is heated to 150 °C for

16 h. After cooling, the excess propionic anhydride is removed under reduced pressure. The crude salt is taken up in ethyl acetate and precipitated with the addition of ether. After sonication, filtration and drying in vacuuo, 3.74 g (81%) of the expected salt is obtained as a brown powder.

6.18. 2-[bis(1-oxopropyl)amino]-[1,1′-biphenyl]-3-sulfonyl chloride, 13 $R_1 = R_2$

To a solution at 0 °C of 3.7 g (8 mmoles) of the triethylammonium salt of 2-[bis(1-oxopropyl)amino]-[1,1′-biphenyl]-3-sulfonic acid in 10 mL of dichloromethane is added, by portions, 2 g (9.62 mmoles, 1.2 equiv) of phosphorus pentachloride. After 4 h of stirring at room temperature, ether is added to the reaction mixture. After decantation, the ether fraction is evaporated and dried in vacuuo. The crude product is taken up in the minimum volume of ether and precipitated with pentane. After cold concentration of the solvents, filtration and drying, 1.4 g (47%) of the product is obtained as a yellow powder.

6.19. 2-[(1-oxopropyl)amino]-[1,1′-biphenyl]-3-sulfonic acid

To 2.49 g (10 mmoles) of 2-amino[1,1′-biphenyl]-3-sulphonic acid is added 7.75 mL (65.5 mmoles; 6.55 equiv) of propionic anhydride. The reaction mixture is heated at 80 °C for 2 h. After cooling, removal of excess propionic anhydride and propionic acid under high vacuum, the crude reaction product is triturated with ether. After filtration and drying under vacuum, 2.87 g (94%) of the crude product is obtained.

6.20. Triethylammonium salt of 2-[(1-oxopropyl)(trifluoroacetyl)amino]- [1,1′-biphenyl]-3-sulfonic acid

To a solution of 2.8 g (9.2 mmoles) of 2-[(1-oxopropyl)amino]- [1,1′-biphenyl]-3-sulfonic acid in 20 mL of dichloromethane is added, at room temperature, 1.94 mL (13.8 mmoles, 1.5 equiv) of triethylamine. The solvent is removed under reduced pressue and the crude salt is dried overnight in vacuuo. To a solution of the salt in 10 mL of dichloromethane, is added 13 mL (92 mmoles, 10 equiv) of trifluoroacetic anhydride. The reaction mixture is refluxed for 3 h. The excess of trifluoroacetic anhydride is removed under reduced pressure. Traces are removed by azeotropic distillation with successive additions of 20 mL of toluene. After drying in vacuuo, 4.61 g (100%) of the crude product is obtained as a brown oil.

6.21. 2-[(1-oxopropyl)(trifluoroacetyl)amino]-[1,1′-biphenyl]-3-sulfonyl chloride, 13, $R_1 \# R_2$

To a solution at 0 °C of 4.61 g (9.2 mmoles) of the crude triethylammonium salt of 2-[(1-oxopropyl)(trifluoroacetyl)amino]- [1,1′-biphenyl]-3-sulfonic acid in 9.2 mL of dichloromethane, is added, at room temperature, by portions, 2.87 g (13.8 mmoles, 1.5 equiv) of phosphorus pentachloride. The reaction mixture is refluxed for 3 h. The reaction product is taken up in ether twice to leave a viscous oil. The ether fraction is evaporated and taken up again in ether. The ether phase is then filtered over Florisil using dichloromethane as the eluent. After removal of the solvents, 2.33 g (60%) of the product is obtained.

6.22. (αS) - α - [(1,1 - dimethylethoxy)carbonyl]amino]-5-methyl-1-(triphenylmethyl)-1*H*-imidazole-4-pentanoic acid

To a solution of 20 g (36.1 mmoles) of (α*S*)-α-[(1,1-dimethylethoxy)carbonyl]amino]-5-methyl-1-(triphenylmethyl)-1*H*-imidazole-4-pentanoic acid methyl ester in 72 mL of methanol is added, dropwise, at room temperature, 43.3 mL (43.3 mmoles, 1.2 equiv) of an aqueous solution of 1N sodium hydroxyde. The reaction mixture is stirred for 24 h at room temperature. The methanol is removed under reduced pressure. The aqueous phase is acidified to pH 3.4 with an aqueous solution of 1N chlorhydric acid and extracted with ethyl acetate. The combined organic phases are washed with brine and dried over magnesium sulfate. After removal under reduced pressure of the solvent, the crude product is taken up in 100 mL of ether. After sonication and filtration, the precipitate is taken up in toluene to remove traces of water and dried in vacuuo to provide 20.7 g (100%) of the crude acid.

6.23. (2*R*,4*R*)-1-[(2*S*)-2-[(1,1-dimethylethoxy)carbonyl]-amino]-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxopentyl]-4-ethyl-2-piperidinecarboxylic acid ethyl ester

To a solution at 0 °C of 6.74 g (12.5 mmoles) of (α*S*)-α-[(1,1-dimethylethoxy)carbonyl]amino]-5-methyl-1-(triphenylmethyl)-1*H*-imidazole-4-pentanoic acid in 100 mL of dichloromethane, is added 3.92 g (13.75 mmoles, 1.1 equiv) of the trifluoroacetate salt of (2*R*,4*R*)-4-ethyl-2-piperidinecarboxylic acid ethyl ester in 50 mL of dichloromethane, followed by 5.75 mL (33 mmoles, 2.64 equiv) of diisopropylethylamine and 5.21 g (13.75 mmoles, 1.1 equiv) of HBTU. The reaction mixture is stirred overnight at room temperature. The reaction solvent is removed under reduced pressure and the crude material is taken up in ethyl acetate. The organic phase is washed successively with aqueous solutions of 1N chlorhydric acid, sodium bicarbonate and brine. The organic layer is dried over magnesium sulfate. After removal of the solvent under reduced pressure, the crude product is purified by chromatography over silica gel using toluene:ethyl acetate as the eluent (from 80:20 to 60:40). 6.96 (79%) of pure product is obtained together with 0.6 g of an impure fraction.

6.24. Hydrochloride salt of (2*R*,4*R*)-1-[(2*S*)-2-amino-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxopentyl]-4-ethyl-2-piperidinecarboxylic acid ethyl ester, 15a

To a solution at 0 °C of 6.96 g (9.86 mmoles) of (2*R*,4*R*)-1-[(2*S*)-2-[(1,1-dimethylethoxy)carbonyl]amino]-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxopentyl]-4-ethyl-2-piperidinecarboxylic acid ethyl ester in 250 mL of benzene is bubbled gazeous chlorhydric acid

for 20 min. After 2 h at 0 °C, the reaction solvent is removed under reduced pressure. The crude reaction product is taken up in ether. After sonication, filtration, and drying in vacuuo, 5.85 g (92%) of the crude hydrochloride is obtained as a white powder.

6.25. Hydrochloride salt of (2*R*,4*R*)-4-ethyl-1-[(2*S*)-5-[5-methyl-1*H*-imidazol-4-yl]-1-oxo-2-[[2-[(1-oxopropyl)-amino][1,1′-biphenyl]-3-yl]sulfonyl]amino]pentyl]-2-piperidinecarboxylic acid ethyl ester, 7

To a solution at 0 °C under nitrogen of 1.42 g (2.15 mmoles) of the di-hydrochloride salt of (2*R*,4*R*)-1-[(2*S*)-2-amino-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxopentyl]-4-ethyl-2-piperidinecarboxylic acid ethyl ester in 25 mL of dichloromethane is added 1 mL (7.09 mmoles, 3.3 equiv) of triethylamine, followed by the dropwise addition of 1 g (2.37 mmoles, 1.1 equiv) of 2-[(1-oxopropyl)(trifluoroacetyl)amino]-[1,1′-biphenyl]-3-sulfonyl chloride in 22 mL of dichloromethane. After 4 h of stirring at room temperature, the reaction solvent is removed under reduced pressure. The crude material is taken up in ethyl acetate. The organic phase is washed successively with an aqueous solution of 1N hydrochloric acid and saturated solutions of sodium bicarbonate and brine. The organic layer is dried over magnesium sulfate. After removal of the solvent, the crude product is dissolved in 150 mL of a 2:1 acetic acid:water mixture. The reaction mixture is refluxed for 4 h. After cooling, the reaction solvents are removed under reduced pressure. The crude material is taken up in ethyl acetate. The organic phase is washed successively with a saturated solution of sodium bicarbonate and brine. The organic phase is dried over magnesium sulfate. After removal of the solvent under reduced pressure, the crude product is purified by chromatography over silica gel using dichloromethane:methanol 90:10 as the eluent. 0.95 g (68%) of the base is obtained and treated with one equivalent of a 0.1 N solution of chlorhydric solution in isopropanol. The resulting salt is purified by chromatography using RP 18, using aqueous 0.01N chlorhydric acid:acetonitrile as the eluent (from 0:100 to 100:0). 0.9 g (90%) of the pure hydrochoride is then obtained as a white lyophilisate. Melting point 104–105 °C; $[\alpha]_D^{20}$ +102° (c = 0,2; MeOH). m/z 652 $(M+H)^+$

^{1}H NMR (500 MHz, Pyridine-d_5) δ (major conformer): 10.20 (broad s, 1H); 10.00 (broad d, IH); 9.60 (broad s, 1H), 8.76 (d, 1H); 8.38 (d, 1H); 7.74 (d, 1H); 7.67 (m, 3H); 7.45 (m, 3H); 4.90 (m, 1H); 4.30 (m, 1H); 3.99 (m, 1H); 2.85–2.75 (m, 4H); 2.50–1.50 (m, 16H); 1.20 (m, 3H); 0.87 (m, 3H); 0.73 (m, 3H).

6.26. (1*S*)-1-[(4-ethyl-1-piperidinyl)carbonyl]-4-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]butyl]-carbamic acid 1,1-dimethylethyl ester

To a solution at 0 °C under nitrogen of 1.67 g (3 mmoles) of (α*S*)-α-[(1,1-dimethylethoxy)carbonyl]amino]-5-methyl-1-(triphenylmethyl)-1*H*-imidazole-4-pentanoic acid in 20 mL of dichloromethane, is added, successively, 0.487 g (3.3 mmoles, 1.1 equiv) of the hydrochloride salt of 4-ethyl-piperidine, 1.36 mL (7.8 mmoles, 2.6 equiv) of diisopropylethylamine and 1.25 g (3.3 mmoles, 1.1 equiv) of HBTU. The reaction mixture is stirred overnight at room temperature. The reaction solvent is removed under reduced pressure. The crude material is taken up in ethyl acetate. The organic phase is washed successively with an aqueous solution of 1N chlorhydric acid, and saturated solutions of sodium bicarbonate and brine. The organic layer is dried over magnesium sulfate. The solvent is removed under reduced pressure and the crude product is purified by chromatography over silica gel using dichloromethane: methanol 99:1 as the eluent. 1.6 g (84%) of pure product is then obtained.

6.27. Hydrochloride salt of 1-[(2*S*)-2-amino-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxopentyl]-4-ethyl-piperidine, 15b

To a solution at 0 °C of 2.4 g (3.78 mmoles) of (1*S*)-1-[(4-ethyl-1-piperidinyl)carbonyl]-4-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]butyl]-carbamic acid 1,1-dimethylethyl ester in 100 mL of benzene is bubbled gaseous chlorhydric acid for 15 min at 0 °C. The reaction mixture is stirred for 1 h at room temperature. The reaction solvent is removed under reduced pressure. The crude material is taken up in ether. After sonication, filtration and drying, 1.8 g (85%) of the crude di-hydrochloride is obtained as a white powder.

6.28. Hydrochloride salt of *N*-[3-[[(1*S*)-1-[(4-ethyl-1-piperidinyl)carbonyl]-4-[5-methyl-1*H*-imidazol-4-yl]butyl]-amino]sulfonyl][1,1′-biphenyl]-2-yl]-propanamide, 8

To a solution at 0 °C under nitrogen of 1.24 g (2.18 mmoles) of the di-hydrochloride salt of 1-[(2*S*)-2-amino-5-[5-methyl-1-(triphenylmethyl)-1*H*-imidazol-4-yl]-1-oxopentyl]-4-ethyl- piperidine in 25 mL of dichloromethane is added, successively, 0.83 g (2.18 mmoles, 1 equiv) of 2-[bis(1-oxopropyl)amino]-[1,1′-biphenyl]-3-sulfonyl chloride and 0.7 mL (5 mmoles, 2.3 equiv) of triethylamine. Stirring is maintained overnight at room temperature. The reaction solvent is removed under reduced pressure. The crude material is taken up in ethyl acetate. The organic phase is washed successively with an aqueous solution of 1N chlorhydric acid, saturated solutions of sodium bicarbonate and brine. The organic layer is dried over magnesium sulfate. After removal of the solvent, 1.62 g (85%) of the crude product is obtained.

1.62 g (1.84 mmoles) of the crude product is dissolved in a mixture of 100 mL of acetic acid and 50 mL of water. The reaction mixture is refluxed for 16 h. The reactions solvents are removed under reduced pressure. The crude material is taken up in ethyl acetate. The organic phase is washed successively with a saturated solution of sodium bicarbonate and brine. The organic layer is dried over magnesium sulfate. After removal of the solvent under reduced pressure, the crude product is purified by chromatography over silica gel. Using dichloromethane:methanol 95:5 as the eluent, 0.77 g of the base is treated with one equivalent of a 0.1N solution of chlorhydric acid in isopropanol. The solvent is

evaporated and the resulting salt is purified by chromatography over RP18 using aqueous 0.01N chlorhydric acid:acetonitrile as the eluent (from 100:0 to 0:100). 0.7 g (55%) of the pure chlorhydrate is then obtained as a white lyophilisate. Melting point 140 °C; $[\alpha]_D^{20}$ +67° ($c = 0{,}2$;MeOH). m/z 580 $(M+H)^+$.

^{1}H NMR (500 MHz, Pyridine-d_5) δ (major conformer): 10.06 (broad s, 1H); 9.45 (broad s, 1H); 8.74 (d, with pyridine, 1H); 8.58, (m, 1H); 7.76 (d, 1H); 7.62 (m, 3H); 7.42 (m, 3H); 4.67 (m, 1H); 2.79 (m, 6H); 2.30–1.32 (m, 14H); 1.00 (m,2H); 0.82 (m, 3H); 0.67 (m, 3H)

6.29. (αS)-5-amino-α-[(1,1-dimethylethoxy)carbonyl]-amino]-2-pyridinepentanoic acid methyl ester

To a solution of 15 g (94.6 mmoles, 1.2 equiv) of 2-chloro-5-nitro- pyridine and 18 g (79 mmoles) of (2*S*)-2-[(1,1-dimethylethoxy)carbonyl]amino]-4-pentynoic acid methyl ester, 27.5 mL (158 mmoles, 2 equiv) of diisopropylethylamine and 0.57 g (3.97 mmoles, 0.05 equiv) of copper bromide in 100 mL of dichloromethane, is bubbled argon for 15 min. 1.11 g (1.58 mmoles, 0.02 equiv) of palladium dichloride bis-triphenylphosphine is then added, still under argon, and the reaction mixture is refluxed for 1 h 30 min. The reaction solvent is removed and the crude material is taken up in 600 mL of ethyl acetate. The organic phase is washed successively with 300 mL of water twice and 300 mL of brine, and dried over magnesium sulfate. After removal of the solvent, the crude adduct is purified by chromatography over silica gel using cyclohexane:ethyl acetate as the eluent (90:10 to 80:20). 22 g (68.8 mmoles) of the crude propargyl adduct is obtained and dissolved in 100 mL of ethyl acetate and 100 mL of methanol. Two spatulas of Raney Nickel are added and the mixture is stirred at room temperature for 1 h. After filtration, the solvents are removed under reduced pressure. The crude material is taken up in 150 mL of methanol and 43.32 g (687 mmoles, 10 equiv) of ammonium formate is added. Argon is bubbled for 10 min. in the reaction mixture. 2.2 g of 10% palladium on charcoal is added and the reaction mixture is refluxed for 2 h 30 min. After cooling, the reaction mixture is filtered over fiber glass and the solvent is removed under reduced pressure. The crude material is taken up in 400 mL of ethyl acetate and the organic phase is washed with 100 mL of brine and dried over sodium sulfate. After evaporation of the solvent, the crude product is purified by chromatography over silica gel using ethyl acetate:methanol as the eluent (98:2 to 94:6). 18.5 g (72%) of the expected product is obtained as an oil.

6.30. (αS)-5-amino-α-[(1,1-dimethylethoxy)carbonyl]-amino]-2-pyridinepentanoic acid, LiCl salt 16

To a solution at 0 °C of 18.5 g (55 mmoles) of (α*S*)- 5-amino-α-[(1,1-dimethylethoxy)carbonyl]amino]-2-pyridinepentanoic acid methyl ester in 100 mL of methanol and 34 mL of water, is added, by portions, 2.56 g (60 mmoles, 1.1 equiv) of lithium hydroxyde. Stirring is maintained overnight at room temperature. The solvent is removed under reduced pressure and the crude salt is treated with 60 mL (60 mmoles, 1.1 equiv) of an aqueous solution of 1N HCl. Water is removed under reduced pressure and traces of water are eliminated by several azeotropic distillations with toluene. The crude product is dried overnight under vacuum. 19.8 g (100%) of the acid is obtained as a 1:1 adduct with lithium chloride.

6.31. [(1*S*)-4-(5-amino-2-pyridinyl)-1-[(4-methyl-1-piperidinyl)carbonyl]butyl]-carbamic acid1,1-dimethylethyl ester

To a solution at 0 °C of 2.84 g (8 mmoles) of (α*S*)-5-amino-α-[(1,1-dimethylethoxy)carbonyl]amino]- 2-pyridinepentanoic acid in 80 mL of dichloromethane and 8 mL of DMF, is successively added, 3.6 mL (20.8 mmoles, 2.6 equiv) of diisopropylethylamine, 1.14 mL (9.6 mmoles, 1.2 equiv) of 4-methyl-piperidine and 2.81 g (8.8 mmoles, 1.1 equiv) of TBTU. The reaction mixture is stirred overnight at room temperature. The solvents are removed under reduced pressure and the crude material is taken up in 300 mL of ethyl acetate. The organic phase is washed successively with saturated aqueous solutions of sodium bicarbonate and sodium chloride. The organic layer is dried over sodium sulfate. The solvent is removed under reduced pressure and the crude product is purified by chromatography using a gradient of ethyl acetate/methanol as the eluent (98:2 to 94:6), 2.69 g (86%) of the pure product is therefore obtained.

6.32. Hydrochloride salt of 1-[(2*S*)-2-amino-5-(5-amino-2-pyridinyl)-1-oxopentyl]-4-methyl-piperidine, 17a

To a solution at 0 °C of 2.6 g (6.5 mmoles) of [(1*S*)-4-(5-amino-2-pyridinyl)-1-[(4-methyl-1-piperidinyl)carbonyl]-butyl]-carbamic acid-1,1-dimethylethyl ester in 40 mL of dichloromethane, is bubbled hydrochloric acid for 1 min.30 and the reaction mixture is stirred 3 h at room temperature. The solvent is removed under reduced pressure and the crude product is dried overnight under vacuum, 2.4 g (100%) of the crude salt is obtained and directly used is the next step.

6.33. Hydrochloride salt of *N*-[3-[[(1*S*)-4-(5-amino-2-pyridinyl)-1-[(4-methyl-1-piperidinyl)carbonyl]butyl] amino]-sulfonyl][1,1′-biphenyl]-2-yl]- propanamide, 9

To a solution at 0 °C of 0.73 g (2 mmoles) of 1-[(2*S*)-2-amino-5-(5-amino-2-pyridinyl)-1-oxopentyl]-4-methyl-piperidine di-hydrochloride in 50 mL of dichloromethane, is successively added, 0.92 mL (6.6 mmoles, 3.3 equiv) of triethylamine and, dropwise, 0.76 g (2 mmoles, 1 equiv) of 2-[bis(1-oxopropyl)amino]-[1,1′-biphenyl]-3-sulfonyl chloride in 10 mL of dichloromethane.The reaction mixture is stirred overnight at room temperature. The solvent is removed under reduced pressure and the crude material is taken up in 200 mL of ethyl acetate. The organic phase is washed successively with 100 mL of saturated aqueous solutions of sodium bicarbonate and. sodium chloride. The organic layer is dried over sodium sulfate. The solvent is removed under reduced pressure and the crude product is taken up in 50 mL of THF and cooled to 0 °C. Gaz-

eous ammoniac is then bubbled for 4 min and stirring is maintained for 2 h at room temperature. The reaction solvent is evaporated under reduced pressure and the crude product dried overnight under vacuum. The crude product is purified by chromatography over silica gel using dichloromethane:methanol 97:3 as the eluent. The free base is treated with 1.1 equivalent of an aqueous solution of HCl 0.1N in isopropanol . After removal of the isopropanol, the salt is purified by reverse phase chromatography using a water:acetonitrile gradient, 0.85 g (69%) of the lyophilised salt are therefore obtained. Melting point 178–182 °C; $[\alpha]_D^{20}$ +71° (c = 0,2;MeOH). *m/z* 578 $(M+H)^+$.

^{1}H NMR (500 MHz, Pyridine-d_5) δ (major conformer): 10.05 (m, 1H); 9.36 (m, 1H); 8.74 (d, with pyridine, 1H); 8.40 (dd, 1H); 7.77 (d, 1H); 7.62 (m, 1H); 7.46 (m, 2H); 7.38 (m, 3H); 7.15 (m, 1H); 6.97 (m, 1H); 4.64 (m, 1H); 3.76 (m, 1H); 2.92 (m, 3H), 2.73 (m, 2H); 2.16 (m, 2H); 1.95–1.22 (m, 9H); 0.78 (m, 3H); 0.75 (m, 3H).

6.34. 4-(difluoromethylene)-1-piperidinylcarboxylic acid-1,1-dimethyl ester

To a solution at 0 °C under Argon of 12 mL (120 mmoles, 2 equiv) of difluorobromethane in 180 mL of triglyme, is added 45.6 mL (252 mmoles, 4.2 equiv) of hexamethylphosphorous triamide (HMPT) in 30 mL of triglyme. The resulting solution is stirred for 30 min at room temperature and cooled again to 0 °C. 11.94 g (60 mmoles) of 4-oxo-1-piperidinecarboxylic acid-1,1-dimethylethylester in 30 mL of triglyme is then added to the reaction mixture. Stirring is maintained for 30 min at room temperature and the reaction mixture is heated to 80°C for 2 h. After cooling, the reaction mixture is poured into 1 l of water. The aqueous phase is extracted three times with 400 mL of pentane. The combined organic layers are washed with water and dried over sodium sulfate. After removal of the solvent under reduced pressure, the crude product is purified by chromatography over silica gel using cyclohexane:ethyl acetate 97:3 as the eluent, 8.5 g (61%) of the expected product is obtained.

6.35. Hydrochloride salt of 4-(difluoromethylene)-1-piperidine

To a solution at 0 °C of 8.5 g (36 mmoles) of 4-(difluoromethylene)-1-piperidinylcarboxylic acid-1,1-dimethylester in 85 mL of benzene, is bubbled for 10 min gaseous chlorhydric acid. After 2 h at 0 °C, the solvent mixture is removed under reduced pressure. The crude product is triturated in ether to afford, after filtration and drying under vacuum, 5 g (83%) of the hydrochloride as a white powder.

6.36. [(1*S*)-4-(5-amino-2-pyridinyl)-1-[4-(difluoromethylene)-1-piperidinyl]carbonyl]butyl]-carbamic acid-1,1-dimethylethyl ester

To a solution at 0 °C under argon of 10 g (28 mmoles) of (α*S*)-5-amino-α-[(1,1-dimethylethoxy)carbonyl]amino]-2-pyridinepentanoic acid, 5.72 g (33.7 mmoles, 1.2 equiv) of 4-(difluoromethylene)-piperidine, 12.74 mL (73 mmoles, 2.6 equiv) of diisopropylethylamine in 140 mL of dichloromethane and 30 mL of DMF, is added 10.8 g (33.6 mmoles, 1.2 equiv) of TBTU. The reaction mixture is stirred for 6 h at 0 °C. The reaction solvent is removed under reduced pressure and the crude material is taken up in 500 mL of ethyl acetate. The organic phase is washed successively with 800 mL of brine, 500 mL of a saturated solution of sodium bicarbonate and 100 mL of brine again. The organic layer is dried over sodium sulfate. The solvent is removed under reduced pressure and the crude product is purified by chromatography over silica gel using ethyl acetate:methanol as the eluent (100:0 to 94:6). 11.5 g (96%) of the expected compound is obtained as a white solid.

6.37. Hydrochloride salt of 1-[(2*S*)-2-amino-5-(5-amino-2-pyridinyl)-1-oxopentyl]-4-(difluoromethylene)-piperidine, 17b

To a solution at 0 °C of 11.2 g (26 mmoles) of [(1*S*)-4-(5-amino-2-pyridinyl)-1-[4-(difluoromethylene)-1-piperidinyl]carbonyl]butyl]-carbamic acid-1,1-dimethylethyl ester in 160 mL of dichloromethane is bubbled, for 1 min 30 seconds gaseous chlorhydric acid. Stirring is maintained at room temperature for 2 h 30 min. The reaction solvent is removed under reduced pressure and the crude salt is dried overnight under vacuum, 10.5 g (100%) of the crude di-hydrochloride is obtained as a white powder and used directly in the next step.

6.38. Hydrochloride salt of *N*-[3-[[(1*S*)-4-(5-amino-2-pyridinyl)-1-[4-(difluoromethylene)-1-piperidinyl] carbonyl]-butyl]amino]sulfonyl][1,1′-biphenyl]-2-yl]-acetamide, SSR182289A, 1

To a solution at 0 °C of 8.7 g (22 mmoles, 1.1 equiv) of 1-[(2*S*)-2-amino-5-(5-amino-2-pyridinyl)-1-oxopentyl]-4-(difluoromethylene)-piperidine-di-hydrochloride in 160 mL of dichloromethane, is added 10.11 mL (72.6 mmoles, 3.2 equiv) of triethylamine. 7 g (19.9 mmoles, 1 equiv) of 2-(diacetylamino)-[1,1′-biphenyl]-3-sulfonyl chloride is then added carefully to the clear solution and stirring is maintained at 0 °C for 2 h 30 min. The reaction solvent is removed under reduced pressure and the crude material is taken up in 800 mL of ethyl acetate. The organic phase is washed successively with 500 mL of brine, 500 mL of a saturated solution of sodium bicarbonate, 200 mL of brine again and dried over sodium sulfate. The solvent is removed under reduced pressure and the crude adduct is taken up in 300 mL of THF. The resulting solution is cooled to 0 °C and bubbled by gaseous ammoniac for 4 min and left with stirring at room temperature for 3 h. The reaction solvent is removed under reduced pressure. The crude product is purified by chromatography over silica gel using dichloromethane:methanol as the eluent. The free base is treated with 1.1 equiv of an aqueous solution of HCl 0.1N in isopropanol and the isopropanol is removed under reduced pressure. The hydrochloride salt is purified by reverse phase chromatography using a gradient of water (pH 2):acetonitrile as the eluent (98:2 to 0:100), 5.85 g (46%) of the hydrochloride salt of the title compound is then obtained as a white lyophili-

sate. Melting point 164–168 °C; $[\alpha]_D^{20}$ +101° (c = 0,2; MeOH).

^{1}H NMR (CD_3OD, 400 MHz) δ: 8.00 (dd, J = 7.9 and 1.7 Hz, 1H); 7.85 (dd, J = 2.7 and 0.4 Hz, 1H); 7.68 (dd, J = 8.9 and 2.8 Hz, 1H) 7.62 (dd, J = 7.9 and 1.6 Hz, 1H); 7.58 d, J = 9.1 Hz, 1H);7.54 (t, J = 7.8 Hz, 1H); 7.48–7.34 (n, 5H) 4.24 (dd, J = 9.5 and 4.1 Hz, 1H); 3.72 (broad dt, J = 12.8 and 4.8 Hz, 1H); 3.5 (broad dt, J = 13.2 and 4.6 Hz, 1H);3.23 (m, J = 12.0 and 3.2 Hz, 1H); 3.04 (m, J = 11.3 and 3.2 Hz, 1H); 2.98–2.78 (m, 2H); 2.21 (broad dt, J = 14.2 and 4.9 Hz, 1H); 2.09 broad dt, J = 14.2 and 4.8 Hz, 1H); 2.01–1.79 (M, 6H) 1.78–1.51 (m,3H); ^{13}CNMR (CD_3OD, 100 MHz) δ 173.0, 171.6, 153.3 (^1J-^{13}C-^{19} F = 282 Hz) 148.5, 145.7, 144.4, 140.2, 139.3, 136.9, 134.7, 131.9, 130.7, 130.2, 129.7, 129.5, 129.2, 128.8, 125.7, 86.0 (^2J-^{13}C-^{19} F = 20.7 Hz) 54.3, 46.8, 43.8, 33.4, 32.7, 26.6, 25.8, 24.7, 23.2. Anal. Calcd for $C_{30}H_{33}F_2N_5O_4S$. 1.28HCl 1.16H_2O C: 54.17H 5.55 N 10.53 Cl 6.82 Found : C 53.96 H 5.24 N 10.41 Cl : 6.65; Karl-Fisher 3.14% H_2O.

To a solution of 30 mg of SSR182289A **1** in 2 mL of methanol in a vial is added 10 mL of a saturated solution of sodium bicarbonate. The reaction mixture is heated at 150 °C for 16 h. The solvents are removed under vacuuo. The crude residue is taken up in 2×5 mL of methanol. After evaporation of methanol, the crude residue is purified by reverse phase chromatography to afford, after lyophilisation, 4 mg of the compound. The compound sample is injected on a 600 E chromatograph (Waters) with a 486 UV detector (Waters) using a Chiralpak AD Daicel column (250×4.6 mm; 5 μM) mobile phase: heptane/isopropanol/DEA 70V/30V 0.1%; flow 0.8 mL/min; detection 254 nm;injection volume 20 μL.

Sample RT (retention time): 14.58 (65.5%) 26.16 (34.5%); Pure SSR182289A **1**: RT 14.67/99.9%); Co-injection : RT : 14.67;26.16

6.39. Coagulation of rat plasma ex vivo—measurement of thrombin time

Male CD Rats weighing 150–200 g were treated with the compound to be tested or its vehicle orally before they were anaesthetised with Nembutal (60 mg/kg, 0.1 mg/kg). Blood was taken in the presence of 3.8% trisodium citrate (1 vol per 9 vol of blood) from the retroorbital sinus. The plasma was prepared by centrifugation at 3600 *g* for 15 min at room temperature. A solution of bovine thrombin was then added. The final concentration of the thrombin was 0.75 NIH units per mL. The time to coagulation (thrombin time) expressed in seconds was noted. The anticoagulant effect was quantified by calculation of the dose (po) which increased the thrombin time by 100% (ED_{100}).

6.40. Determination of inhibitor constants for human enzymes[12]

The inhibitor constants (K_i) were determined for inhibition by SSR182289A **1** and Melagatran **5a** of a series of human enzymes.

Chromogenic substrate assays were performed using a Labsystems IEMS (Cergy Pontoise, France) microtitre plate reader and Biolise software. K_i values were calculated according to the method of Dixon. In each assay the compound was tested at a minimum of seven concentrations in duplicate in order to obtain an inhibition curve. Two different substrate concentrations were used (four in the case of SSR182289A **1**/thrombin). Assays were performed according to the following general procedure. In a 96-well microtiter plate, 25 μL of inhibitor solution or buffer was added to 50 μL of substrate. A volume of 25 μL of enzyme solution was added just before the plate was placed in the microtiter plate reader for 1 h at 37 °C. The hydrolysis of the substrate yields *p*-nitroaniline which was continuously monitored spectrophotometrically at 405 nm. Maximal initial reaction rates were calculated and expressed as mOD/min. Curve fitting (Dixon plot of $1/V_{max}$ versus inhibitor concentration) was performed by linear regression analysis to calculate the K_i value.

6.41. Determination of inhibition of thrombin generation in plasma[12]

Thrombin generation experiments were carried out in defibrinated plasma obtained by mixing an aliquot of platelet-poor plasma with ancrod (50 U/mL), letting a clot form for 10 min at 37 °C and keeping the clotted plasma at 0 °C for 10 min. The fibrin thus formed was discarded before thrombin generation determination. 100 μL of a chromogenic substrate (S2222 0.25 mM, which is converted by thrombin sufficiently slowly and yet shows reasonable specificity for thrombin), then 100 μL of recombinant tissue factor and 100 μL of Ca^{++} buffer (Tris HCl 0.05 M, NaCl 0.1 M, 100 mM $CaCl_2$, pH 7.35, 0.05% ovalbumin) were added to a disposable plastic microcuvette. Following this, 100 μL of buffer (Tris HCl 0.05 M, NaCl 0.1 M, pH 7.35, 0.05% ovalbumin) containing either SSR182289A **1** or Melagatran **5a** was added to the mixture. The reaction was started at zero time by adding defibrinated plasma. The reagents were pre-warmed to 37 °C and the cuvette was thermostatically controlled at that temperature during the measurement. The optical density at 405 nm was recorded at the rate of 10 measurements per min using a spectrophotometer. From the obtained curve, Endogenous Thrombin Potential (ETP) was calculated. Percentage inhibition was then calculated according to the formula: (ETP control−ETP in presence of compound)/ETP control. Results were expressed as IC_{50} values.

6.42. Inhibition of platelet aggregation[12]

Platelet aggregation studies were performed in human platelet rich plasma (PRP). Human blood was collected from the cubital vein of volunteers, following ethical committee approval, at the Centre de Transfusion Sanguine, Toulouse, France. Blood was collected using 3.8% sodium citrate as anticoagulant (one vol citrate to nine vol blood). PRP was prepared by centrifugation of blood at 500 *g* for 10 min. The remainder was centrifuged at 1500 *g* for 10 min to prepare platelet poor plasma.

Platelet aggregation was measured at 37 °C, according to the turbidimetric method of Born and Cross in an aggregometer (Dual Aggregometer, Chrono-Log, Haverton, PA.) after activation of PRP by 0.2 U/mL thrombin for human. Aggregation with thrombin was performed with a PRP diluted four times in NaCl 0.9%. Drugs were added 1 min before activation, in distilled water or DMSO solution at final concentration of 0.01%. The extent of aggregation was estimated quantitatively by measuring the maximum amplitude above baseline level.

The anti-aggregatory activities of SSR182289A **1** and Melagatran **5a** were calculated as percentage inhibition of aggregation compared with the vehicle control. Results are expressed as IC_{50} values.

6.43. Rat venous (wessler) thrombosis model[13]

Fasted male CD rats (300–410 g, Charles River, France) were anaesthetised with pentobarbitone sodium (60 mg/kg, ip) and placed on a heated jacket to control body temperature (Harvard, Les Ulis, France). The left jugular vein was cannulated for intravenous injection of drugs. The abdominal vena cava was exposed and two silk sutures, 1 cm apart, were placed around the vessel to form a snare. Thrombus formation was induced by the intravenous injection of 20 μg/kg rabbit thromboplastin (La Technique Biologique, Paris, France) into the left femoral vein followed 10 seconds later by tightening the two snares around the vena cava to induce blood stasis. Stasis was maintained for 15 min., after which time, the thrombus was removed and immediately weighed. SSR182289A **1** or Ximelagatran **5b** or vehicle were administered as single oral doses 60 min before injection of thromboplastin.

6.44. Rat arterio-venous shunt model (tissue factor induced) [13]

Fasted male CD rats (260–410 g, Charles River, France) were anaesthetised with sodium pentobarbital (60 mg/kg ip). The left jugular vein and the right common carotid artery were cannulated with a 8 cm long polyethylene cannulae (Biotrol n° 3, Paris, France) mounted on 25/8 needles. The shunt was assembled by connecting the two cannulae with a slightly curved 6 cm long polyvinyl chloride tube (internal diameter = 3 mm) containing a 5 cm long cotton thread impregnated with tissue factor [threads preincubated 5 min in a 35 mg/mL thromboplastin solution (La Technique Biologique, France) and then dried]. The extracorporal circulation was maintained for 5 min, during which time a thrombus adhered to the cotton thread. The shunt was then removed, the thread with its associated thrombus was withdrawn and immediately weighed. The thrombus wet weight was determined by subtracting the average weight (5 mg) of a 5 cm long cotton filament from the value obtained. SSR182289A **1**, Ximelagatran **5b** or vehicle were administered as single oral doses 30 min before shunt assembly. In a separate study designed to measure the duration of the antithrombotic effect, SSR182289A 1 (10 mg/kg po) or Ximelagatran **5b** (10 mg/kg p.o.) or vehicle were administered, each, to four groups of animals at different times (30, 60, 120 or 240 min) before shunt assembly.

6.45. Rat arterial thrombosis model[13]

Fasted male CD rats (420–510 g, Charles River, France) were anaesthetised with sodium pentobarbital (60 mg/kg followed by 6 mg/kg/h, ip) and thermoregulated by use of blankets (Harvard homeothermic blanket control unit, Les Ulis, France). A segment (approximately 1 cm long) of the left carotid artery was exposed and fitted at the distal end with an appropriately sized Doppler flow probe. Thrombosis was induced by applying an electrical current (3 mA DC) to the external arterial surface using a DC stimulator (Sanofi-Synthélabo) for 2 min. Blood flow velocity was measured using a Doppler flowmeter (Triton Technology—system 6—Model 200, San Diego, USA) and recorded on a chart recorder (Graphtec - DMS 1000, Bioseb, Antony, France). Blood flow was recorded for 60 min post-lesion. When the flow declined to zero, the time in min to thrombus formation was noted. SSR182289A 1, Ximelagatran 5b or vehicle were administered as single oral doses 30 min before thrombus induction.

Acknowledgements

The authors would like to thank Dr. T. Purcell and Dr J.C. Muller for their support in the critical early days, Dr. L. Zard, Dr. D. Legroux and colleagues (Chemical Development, Porcheville) for very fruitful discussions and for providing large amounts of intermediates, Dr Ph. Poirier, M. Sebban and colleagues (DARA, Rueil) for their help in structure determinations. Also special thanks to C. Lecoffre, C. Girardot, D.Girard, M.C. Charles, C. Gaich, A Lalé, S. Lochot, I. Lechaire, L. Millet, M. Sainte-Marie and to all the chemists of the Cardiovascular-Thrombosis Department who contributed to the programme, Dr. G. Georges for molecular modelling and Mrs. Juliana Mersovska for technical assistance during the preparation of the manuscript.

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